Claim amendments

Support for new claims 33-37 can be found *inter alia* in claims 2 and 4 as originally filed, in the specification at page 43 lines 1-8, and in Example 4 (page 50 ff.). Accordingly, no new matter is added to the disclosure as a result of entering these amendments.

Rejection under 35 USC § 112 ¶ 1

Claims 1-6 stand rejected as not being adequately described in the specification to convey that applicants were in possession of the claimed invention. Claims 1-6 also stand rejected as not being enabled for making tissues devoid of Galα(1,3)Gal in any strain of ovine. The Office Action raises four concerns:

1. Whether heterozygous animals will have antibody-detectable Galα(1,3)Gal on their tissues;
2. Whether the application describes tissue devoid of Galα(1,3)Gal of all tissue types falling within the scope of the claim;
3. What the effect will be of the backgrounds of different strains of ovine (with respect to the issues raised in the article by C.C. Linder, Lab Animal 30:34, 2001).
4. Whether homologous recombination will work with strains of ovine that are different from Finn Dorset.

Applicants respectfully submit that the specification complies with the requirements of 35 USC § 112 ¶ 1.

The specification describes for the first time the isolation and sequencing of the ovine α(1,3)galactosyl-transferase (α1,3GT) gene. It further describes the use of targeting constructs of the α1,3GT gene to make cells and animals that do not express the α1,3GT enzyme, and consequently do not have the Galα(1,3)Gal determinant on any of their cells. The specification provides evidence of successful targeting with non-isogenic DNA, and fully places the claimed invention in the hands of the skilled reader.

With respect to issue 1: The specification indicates on page 36, line 25, that the expression of the Galα(1,3)Gal epitope is autosomal dominant. The skilled reader will readily appreciate why this is so. Cells express N-acetyl lactosamine, the precursor substrate for the α1,3GT enzyme. The enzyme adds galactose in the α(1,3) position of the precursor to form the Galα(1,3)Gal determinant (specification, pages 1 & 2).

Accordingly, Galα(1,3)Gal will only be absent if the cells don't express any α1,3GT — i.e., the α1,3GT gene must be inactivated on both haplotypes (a homozygous knockout). A cell or animal that is inactivated for α1,3GT on just one allele will still have Galα(1,3)Gal on their cells at a density that would often still be antibody detectable, and a potential problem for xenotransplantation. Nevertheless, cells that have a knockout of only one locus are useful, because they can be used to make heterozygous knockout animals, which in turn can be cross-bred to produce homozygous knockout animals.

The Office Action cites a paragraph from the article by Tearle et al. (Transplantation 61:13, 1996) as standing for the proposition that homozygous knockouts are reduced by only 60% in the amount of reactivity by anti-Galα(1,3)Gal antibody (page 15, col. 2 ¶ 3). This is not correct. In the preceding paragraph on page 15, Tearle et al. report that homozygous knockout (*GalT-/-*) mice do not express *any* Galα(1,3)Gal epitope, as detected by the IB_4 lectin. The antibody test is done using whole human serum, for which anti-Galα(1,3)Gal activity represents about 60% of the total anti-xenoantibody (page 16, col. 2 ¶ 4). Thus, when Galα(1,3)Gal is removed from the cells, reactivity of the anti-Galα(1,3)Gal activity is eliminated. But in the mouse, antibody activity remains due to reactivity against *other* xenoantigens, which accounts for the residual 40% reactivity.

Claim 1 of this application does not require that the ovine tissue be devoid of xenoantigens of any kind . This makes sense, since the cell is still an ovine cell. However, tissue from a homozygous Galα(1,3)Gal knockout will be essentially devoid of the Galα(1,3)Gal determinant, meeting the requirement of the claim.

With respect to issue 2: Homozygous α1,3GT knockout animals made in the manner described in the specification will not have an operable α1,3GT gene anywhere in their body. Accordingly, precursor substance will not be converted to Galα(1,3)Gal in any tissue.

Tissue of any kind harvested from these animal will be essentially free of the Galα(1,3)Gal epitope.

With respect to issue 3: The Linder article describes different phenotypes that may result by knocking out particular genes in inbred strains of mice.

One issue raised in the article is that inactivation of a gene may generate different phenotypes in particular inbred strains of mice. Table 2 shows that knocking out the gene for IL-2 can cause splenomegaly, inflammatory bowel disease, or generalized autoimmune disease, depending on the genetic background. A similar observation is made for *ob/ob* obese mice, which have a homozygous mutation in the leptin gene.

The target genes in these studies are both endocrine molecules (IL-2 and leptin) which mediate a complex response pathway between different cells. In contrast, the present invention is directed at inactivating a gene that puts a terminal sugar residue onto the carbohydrate substrate N-acetyl lactosamine, which all ovine animals express. Accordingly, no inter-strain variation is expected.

Another issue raised in the Linder article is that a cross-over event that occurs during breeding may separate a mutant gene from the phenotype being used to follow the breeding, if the phenotype is not directly encoded by the mutated gene. This is not a concern for the present invention, because the presence of an inactivated target gene can be detected more directly — by PCR analysis (Figures 16 and 17), or by antibody binding.

As indicated in the Background section of this application (page 4), U.S. Patent 5,849,991 (Bresatch) describes DNA constructs based on the mouse α1,3GT sequence. It is reported that mice have been bred that are homozygous for inactivated α1,3GT, resulting in lack of expression of Galα(1,3)Gal epitope, as determined by specific antibody. Thus, the previous experience is that α1,3GT knock-out animals reliably breed towards absence of the Galα(1,3)Gal epitope, as expected.

With respect to issue 4: The claims stand rejected as not enabled for strains of sheep other than Finn Dorset, which is the model provided in some of the working examples.

The Office Action apparently assumes that genomic sequences will be sufficiently different between different strains to prevent homologous recombination from occurring. In fact, use of non-isogenic DNA within the same strain may reduce the frequency *but not the feasibility* of homologous recombination.

- Riele et al. (Proc. Natl. Acad. Sci. USA 89:5128, 1992) report that recombination was less efficient *but still possible* using a targeting vector from another mouse strain that contained base pair substitutions, small dilutions and insertions, and a polymorphic repeat.
- Mocikat et al. (Immunol. 84:159, 1995) report successful recombination between immunoglobulin sequences of different mouse strains.
- Zhou et al. (Mammalian Genome 12:772, 2001) report homologous recombination at reduced frequency using non-isogenic targeting vector form the μ-opioid receptor.

Accordingly, it is reasonable to expect that homologous recombination using non-isogenic ovine vectors, with or without reduction in recombination frequency. As an alternative, the reader can manufacture targeting vectors that are isogenic for other strains of ovine using the sequence data for the sheep cDNA and amplification primers provided in the specification (Examples 2 & 3).

The Office Action indicates that the specification shows a failure to target α1,3GT genes in black Welsh mountain sheep, and is only successful in Finn Dorset sheep. This is incorrect. The last paragraph of Example 4 indicates that a particular vector targeting Exon 8 or 9 of the α1,3GT gene in Black Welsh Mountain fibroblasts did not cause homologous recombination to the extent tested. But the p0054 vector targeting Exon 4 was used successfully to create α1,3GT knockout cells. This is not surprising, since the genomic α1,3GT sequence was originally obtained from a Black Welsh Mountain DNA library (Example 2).

In fact, the specification provides direct confirmation that targeting of the α1,3GT gene using non-isogenic DNA can easily be done based on this disclosure. The p0054 vector was

used to produce 18 colonies of α1,3GT deleted cells of the Finn Dorset line, which is non-isogenic to the p0054 vector (Example 5).

Withdrawal of all rejections under 35 USC § 112 is respectfully requested.

Conclusion

These papers are believed to place the application in condition for allowance, and a rapid Notice of Allowance is requested.

In the event that the Examiner determines that there are other matters to be addressed, applicants hereby request an interview by telephone.

Should the Patent Office determine that a further extension of time or any other relief is required for further consideration of this application, applicant hereby petitions for such relief. The Assistant Commissioner is hereby authorized to charge the cost of such petitions and other fees due in connection with the filing of these papers to Deposit Account No. 07-1139.

Respectfully submitted,

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ANIMAL TISSUE FOR XENOTRANSPLANTATION

09/593,316
730/002

Claims under Examination:

1. Ovine tissue devoid of antibody-detectable Galα(1,3)Gal determinants.

2. The tissue of claim 1, which is selected from the group consisting of lung tissue, kidney tissue, liver tissue, cardiac tissue, pancreatic tissue, and ocular tissue.

3. Isolated ovine cell or tissue that expresses glycosyl transferase enzymes but does not detectably express α(1,3)galactosyltransferase(α1,3GT).

4. An ovine cell which is heterozygous or homozygous for inactivation of an α1,3GT gene.

5. The cell of claim 4, produced by transfer of a nucleus from a donor cell heterozygous or homozygous for inactivation of an α1,3GT gene, to an enucleated recipient cell.

6. An ovine animal that is homozygous for inactivation of an α1,3GT gene.

33. *(New)* The cell of claim 4, which is a fibroblast.

34. *(New)* The cell of claim 4, which is a kidney cell.

35. *(New)* The cell of claim 4, which is a hepatocyte.

36. *(New)* The cell of claim 4, which is a cardiac cell.

37. *(New)* The cell of claim 4, which is an islet cell.

Claims Withdrawn from Examination:

7. A polynucleotide construct effective for inactivating an α1,3GT gene in an ovine cell.

13. A method for producing an ovine that is homozygous for inactivation of an α1,3GT gene, comprising providing an ovine embryo of cells according to claim 4, engrafting the embryo into the uterus of a female, birthing an ovine with an inactivated α1,3GT gene from the engrafted female, and if the birthed ovine has the α1,3GT gene inactivated on only one allele, then mating it with another ovine with an inactivated α1,3GT gene, thereby producing an ovine that is homozygous for inactivation of the α1,3GT gene.

14. A method for producing an isolated ovine cell that expresses glycosyl transferase enzymes but does not detectably express α1,3GT, comprising isolating the cell from an ovine homozygous for inactivation of an α1,3GT gene according to claim 6.

15. A method for producing ovine tissue devoid of antibody-detectable Galα(1,3)Gal determinants, comprising harvesting the tissue from an ovine homozygous for inactivation of an α1,3GT gene according to claim 6.

16. A method of xenotransplantation, comprising transplanting tissue devoid of antibody-detectable Galα(1,3)Gal determinants according to claim 1 into a mammal having circulating antibody against Galα(1,3)Gal determinants.

17. An isolated polynucleotide that comprises a sequence of at least 30 consecutive nucleotides with at least one of the following properties:

a) it is contained in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13;

b) it is contained in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061; but not in λ-phage or any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13; or

c) it hybridizes under stringent conditions to a polynucleotide with the sequence in SEQ. ID NO:1 or any of SEQ. ID NOs:14 to 25, but not to a polynucleotide with the sequence in any of SEQ. ID NOs: 3, 5, 7, 9, 11, and 13

22. An isolated polypeptide that comprises a sequence of at least 10 consecutive amino acids with at least one of the following properties:

a) it is contained in SEQ. ID NO:2 but not in any of SEQ. ID NOs: 4, 6, 8, 10, and 12;

b) it is encoded in phage **B**, **C** and **G** deposited under Accession Nos. NCIMB 41056, 41059, 41060, and 41061., but not encoded in λ-phage or present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12; or

c) it is at least 80% identical to 15 consecutive amino acids contained in SEQ. ID NO:2, wherein said sequence is not present in any of SEQ. ID NOs: 4, 6, 8, 10, and 12

27. An isolated polynucleotide comprising a sequence encoding a polypeptide according to claim 22.

28. An isolated polyclonal antibody or a monoclonal antibody that binds specifically to a polypeptide with the sequence SEQ. ID NO:2 but not to a peptide with the sequence present in any of SEQ. ID NOs: 4, 6, 8, or 10.

29. An assay method for determining α1,3GT expression by a cell, comprising contacting a polynucleotide according to claim 17 with the cell or with mRNA or cDNA obtained from the cell, detecting any hybrids that form as a result, and correlating presence of the hybrids with expression of α1,3GT by the cell.

30. A method for producing the antibody specific for sheep α1,3GT, comprising immunizing an animal or contacting an immunocompetent particle with a polypeptide according to claim 22.

31. A method for preparing a Galα(1,3)Gal determinant, comprising contacting a galactose acceptor saccharide with the polypeptide of claim 26 in the presence of UDP-galactose.

32. An assay method for determining α1,3GT in a sample, comprising preparing a reaction mixture comprising the sample and an antibody according to claim 28 under conditions that permit the antibody to complex with α1,3GT, and correlating any complex formed with the presence or amount of α1,3GT in the sample.